THE RESPONSE IN GENERAL

The presently pending independent claims each recite a group of binding materials which are strongly hydrophobic. These materials are not taught as being used in the cited art. Thus, the claimed invention is not taught by the cited art. The cited art does not suggest the use of the binding materials now claimed or recognize the improved results which could be obtained with such materials. Thus, the cited art does not render the claimed invention obvious. The Examples on pages 16-19 demonstrate the improved results obtained with applicants' method using the particular binding materials now claimed.

The presently pending claims are directed to improved methods for concentrating and desalting oligonucleotides following a purification procedure. The use of materials that strongly adhere to nucleic acids, such as poly(styrene-divinylbenzene), allows the use of an unbuffered aqueous solution to wash the salt from the column. Following rinsing, the nucleic acids can be eluted from the column using a compatible non-toxic aqueous unbuffered organic solvent nucleic acid being concentrated in a desalted solution. The desalted solution can then be easily lyophilized to yield the pure, desalted nucleic acid in a dried form.

The method of the present invention is effective at removing non-volatile salts, such as sodium, as well as volatile salts such as ammonium. In the present invention, the high affinity column *(e.g.,* the polystyrene-divinylbenzene column) bearing the oligonucleotide is washed to achieve an eluant with essentially background levels of conductivity. When the oligonucleotide is eluted using the organic phase in water, no salt remains on the column, and thus no further purification is necessary to remove the salt.

The Sep-Pak C18 cartridge procedure that is cited in each of the references is geared toward the removal of side products generated by the cleaving and deprotection of a synthetically prepared oligonucleotide. This procedure must use a volatile salt during the washing step and the elution step in order to remove the salt during lyophilization. Since the salt used must be volatile in order to be removed during the vacuum drying, this protocol is quite limited in the variety of cations that can be used directly in the procedure.

In the cited methods, using a C18 stationary phase, the salt acts as a ion-pairing reagent and, when removed, the oligonucleotide does not have enough affinity to remain bound to the C18 stationary

phase. Thus, unlike the present invention, the oligonucleotide would elute before the salt had been removed, these binding medium cannot be washed to rid the salt before eluting the oligonucleotide. The same premature elution problem can also be seen on C4 and phenyl reverse materials.

Moreover, if a counterion is desired other than those associated with volatile salts, the oligonucleotide prepared with the use of a Sep-Pak C18 column must be exchanged by salt precipitation from a solution with the desired counterion present, or by cation exchange chromatography following use of the Sep-Pak C18 column. The method of the present invention does not require such an additional step, and in fact the cation may be exchanged in situ on the column. Thus, it provides a faster and more concise method of providing an oligonucleotide with a specified cation.

Obviousness under §103(a) over U.S. Pat Nos. 5,929,226 and 5,275,946

Claims 1-4 and 6-21 were rejected as being obvious in view of U.S. Pat Nos. 5,929,226 and 5,275,946. This rejection is traversed as applied, and as may be applied to the presently pending claims.

To establish prima facie obviousness, 1) a reference or combination of references must suggest the combination of Applicants' invention or motivate one of skill in the art to modify the reference to achieve Applicants' invention; 2) the prior art must teach or suggest all claim limitations; and 3) there must be a reasonable expectation of success.

Both of the cited patents teach the use of a Sep-Pak C18 cartridge to purify oligonucleotides. Neither of these references teaches the use of a binding substrate with an affinity of polydivinylbenzene, poly(styrene-divinylbenzene), polystyrene copolymers, polyethylene, or polypropylene, nor do they suggest the benefits of doing so, i.e. the one step concentration and desalting. In fact, the art cited by the Examiner teaches the use of columns such as a Sep-Pak C18 column; Applicants explicitly state in the specification that "[o]ther reverse-phase solid phases (such as C4 and C18) and hydrophobic interaction chromatography phases do not absorb the nucleic acid sufficiently well to allow the use of unbuffered water to wash away the salt to the desired low level" Page 14, lines 19-23." Since these references do not teach the use of these higher affinity binding mediums, nor suggest the benefits of doing so, the present invention is not *prima facie* obvious in view of this art, as they 1) do not teach or suggest each

limitation of Applicants claims and 2) would not motivate one of skill in the art to substitute the claimed binding mediums in the cartridge.

Thus, Applicants respectfully request withdrawal of the §103 rejection over the U.S. Pat Nos. 5,929,226 and 5,275,946 and allowance of the presently pending claims.

Obviousness under §103(a) over The Lineberger Nucleic Acids Core Facility web site

Claims 1-4 and 6-21 also stand rejected under §103 over the Lineberger Nucleic Acids Core Facility web site.

The Lineberger Nucleic Acids Core Facility web site describes a method for concentrating nucleic acids. This procedure, similar to those used in U.S. Pat Nos. 5,929,226 and 5,275,946, also used a C18 Sep-Pak. This site does not teach the use of higher affinity binding mediums such as claimed by applicants nor suggest any potential benefits of substituting a binding medium with a higher affinity than the C18 medium. Since this site does not teach nor suggest each limitation of Applicants' invention as claimed, and since nothing in the site would motivate one of skill in the art to substitute the claimed binding mediums in the cartridge, Applicants' invention is also not *prima facie* obvious in light of this site.

Thus, Applicants respectfully request withdrawal of the §103 rejection over the Lineberger Nucleic Acids Core Facility web site and allowance of the presently pending claims.

Rejection of claims 1-4 and 6-21 over Art in the Background

None of the art cited in the background top the specification teaches nor suggests the use of materials with very high binding affinities, e.g., binding affinities greater than a C18 substrate. Thus, for the reasons given above, none of the cited art would render the claims as presently pending obvious under §103.

Other considerations for non-obviousness

Objective indicia of non-obviousness include evidence that the claimed subject matter had gone undeveloped by others, despite a motivation for doing so. Thus, evidence of a long-felt need that had

not been satisfied until the patentee's invention of the claimed subject matter provides some indication that the patentee's claims may not have been obvious, particularly if others had attempted to meet the need, but had failed. Applicants would like to reiterate the need for improved methods that was discussed in Paper No. 4, the response to the first Office Action. Others in the field were attempting to find a better and more efficient method for desalting and concentrating oligonucleotides and/or monomers, and were met by only a limited success. Thus, the methods of the current invention, which are a distinct improvement over the other available methods, is not obvious under §103.

CONCLUSION

The presently pending claims have been amended to place the claims in better condition for allowance. No new matter was believed to be added by any of the amendments made herein. For the reasons given herein, and for the reasons of record in Paper No. 4 such as long felt need in the art, the claims are not obvious under §103. Accordingly, Applicants respectfully request allowance of the currently pending claims.

If Examiner feels it would expedite prosecution to speak with the undersigned attorney, please feel free to contact her directly at (650) 833- 7716.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 and 1.17 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-0815.

Respectfully submitted,
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